Relationships Between Hydrogen Ion, Hydroxyl Ion and Salt Concentrations and the Growth of Seven Soil Molds

DUPLICATE

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Some Relationships Between Hydrogen Ion, Hydroxyl Ion and Salt Concentrations and the Growth of Seven Soil Molds*

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Comparatively few data have been recorded relative to the effect of hydrogen ion concentration on the development and activities of molds and other fungi, altho such records have been secured for the bacteria. For both groups much more work has been accomplished on the effect of titratable acidity. The importance of molds in transforming plant food in the soil has made advisable the securing of such data. The present paper records the results of a study of the limiting and optimum hydrogen and hydroxyl ion concentrations and the limiting concentrations of several salts as related to the growth of seven different molds isolated from the soil.

HISTORICAL

A number of investigators have shown that molds are of great importance in soils. Only a few of the results obtained need be given to prove their value and hence the need for more study of their characteristics.

Waksman (36) has isolated over 200 species belonging to 42 genera from various soils and says that all are active in decomposing organic matter and liberating ammonia. Jensen (20) isolated 132 species from soils. McLean and Wilson (28) showed that the mold flora of soils is active in ammonification, more active, indeed, than the bacterial flora. Potter and Snyder (30) proved that molds inoculated into sterile soils give nearly as much carbon dioxide as if the natural soil flora were used as the inoculum. McBeth and Scales (27) found that over 40 percent of the mold colonies isolated from soils have the ability to decompose cellulose. Brown (1) concluded that molds are of importance in soils, not only because they prepare nitrogenous material for the use of higher plants, but also because they make mineral elements available. Hall, Miller and Gimingham (14) attribute the decline in fertility on plots made acid by continued use of NH_4Cl and $(NH_4)_2SO_4$ to the repression of the normal bacterial activities of the soil and the encouragement of the growth of molds.

Several workers, in studying the characteristics of molds, have investigated the effect of titratable acidity upon their growth.

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It is the general opinion among those who have studied fungi that their growth and most of their activities are favored by an acid reaction.

Thom (34) in a study of species of *Penicillium,* found the range of tolerance to be from 2 c. c. normal NaOH to 5 c. c. of normal lactic acid per 10 c. c. of media. Few of his molds grew in alkaline media and he concluded that the optimum range was from neutral to tenth normal acid. Clark (6) in a study of five molds found that on the average $^{7}\%_{2048}$ normal HCl caused injury, $^{230}\%_{2048}$ normal HCl inhibited growth, and $^{614}\%_{2048}$ normal HCl killed the molds. In comparing molds with phanerogams, he states that N/33 HCl killed the former while N/2740 HCl killed the latter. One mold, *Sterigmatocystis nigra V,* required N/2 HCl to kill it, N/4 HCl to inhibit its growth and N/1024 HCl to injure it. By growing the molds in KCl solutions of the same or greater normality he showed that the hydrogen ion and not the chlorine ion was the injurious element. As a result of this study he concluded that the hydrogen ion is 32 times as toxic as the chlorine ion. He also found that the hydroxyl ions are much more toxic than the hydrogen ions.

Fellers (10) found molds growing on agar plates when the medium had been acidified with 40 or 50 c. c. of normal HCl per liter. He concluded that an acidity produced by 10 to 15 c. c. of normal HCl per liter was best for counting molds on plates. An acid soil which he used in his experiments gave a development of more mold colonies than two neutral soils. He also observed a *Penicillium* growing in N/10 HCl. Stevens (33) reported that spores of *Macrosporium* germinated in N/50 HCl and in N/100 NaOH and of *Penicillium crustaceum* in N/50 HCl and in N/20 NaOH.

A few data are available on the effect of reaction on the physiological activities of molds. Potter and Snyder (30) noted that additions of $CaCO_3$ to a soil having a lime requirement determined by the Veitch method of 1,540 pounds per acre caused no marked increase or decrease in the amount of carbon dioxide given off by mold action. McLean and Wilson (28) reported that the ammonifying power of *Trichoderma koningi* was depressed by acid phosphate but increased by $CaCO_3$, while *Rhizopus nigricans* produced more ammonia with additions of acid phosphate and neither a greater or smaller amount with $CaCO_3$. With a *Penicillium,* the addition of $CaCO_3$ decreased the ammonia produced by about one half while acid phosphate gave a large increase.

Kopeloff (22), working with fungi, found that the greatest production of ammonia occurred in neutral soils or those made acid with HCl up to 2,000 pounds lime requirement per acre and that additions of NaOH decreased materially the ammonia

production of several molds. Using H_2SO_4 and $CaCO_3$ he obtained similar results.

The workers, whose results have thus far been referred to, have considered only titratable acidity. Since Fuller (12) in 1895 introduced his scale for adjusting the reaction of media according to titratable acidity, it has been used as a standard in bacteriological work. It is universally accepted that media must be adjusted as to reaction if reproducable results are to be obtained. More recent work, however, has shown that Fuller's scale, except in special cases, is altogether inadequate. The study of culture media for bacteria has shown quite definitely that the titratable acidity is not the factor that affects bacterial development. Rather it is the actual acidity or the concentration of ionized hydrogen present which causes erratic behavior or inhibits the growth of the bacteria.

Clark (6, (2))* points out that the scale fails in that phenolphthalein in culture media gives no true end point, that the standard color of the indicator has no significance, that in adjusting different media to the same degree on the scale, very wide differences in hydrogen ion concentration are arrived at and that the titer obtained in hot media is not applicable when the media are at incubation temperatures. He says, "It may be seriously suggested that too strong adherence to old titration methods is a hindrance to progress." Fennel and Fisher (6) state that for the titration of culture media the use of phenolphthalein as an indicator to determine the total acidity is rapidly becoming obsolete since there is no constant inter-relationship between hydrogen ion concentration readings and phenolphthalein titration results, any changes in technic changing the relationship.

Norton and Hsu (6) conclude that the disinfecting power of an acid is approximately proportional to the hydrogen ion concentration. Winslow and Lockridge (6) report that the mineral acids, HCl and H_2SO_4, are fatal in concentrations at which they are highly dissociated, their lethal action being proportional, not to the normality strength of the acid, but to the number of free hydrogen ions per unit volume. Wright (6), reporting on a study of disinfectants, says that the greater part of the difficulty in securing concordant results lies in the present methods of adjusting the acidity, there being a marked relationship between the hydrogen ion concentration of the culture medium and the resistance of the organisms tested to the action of disinfectants.

Many others have reported similar conclusions and a great amount of work has been done to determine the effect of hydro-

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* All references numbered (6) may be found in Clark's "The Determination of Hydrogen Ions." In case an author is cited more than once in Clark's bibliography the number following the (6) indicates the reference alluded to.

gen ions on bacteria. It is not necessary here to refer to this work except to say that it has been found that most organisms have well defined limits of hydrogen ion concentrations in which they will grow, as well as an optimum concentration at which they grow best. Similar limiting and optimum hydrogen ion concentrations have also been found for enzyme activity. The results of some of the work on bacteria, yeasts and enzymes will be compared with the results obtained in the experiments in a later part of the paper.

Very few data, however, are available on the effect of hydrogen ion concentration on molds. Currie (6) reports that the critical P_H value for *Aspergillus niger* is from 1.6 to 1.4. He states that the titratable acidity in a citric acid solution of P_H=1.4 is sixty-two times that of a solution containing HCl of the same P_H value and same inhibiting effect. One culture of *Aspergillus* made a considerable growth in 40 percent citric acid. Meacham (6) reports that with four wood destroying fungi, *Lenzites sepiaria, Fomes roseus, Coniophora cerebella* and *Merulius lachrymans,* the effects of acidity are variable but that as an average, a P_H of 2.75 did not injure growth while P_H 1.7 inhibited it completely. He shows that the curves for weight of growth plotted against the initial hydrogen ion concentration of the medium in which the growth occurred, are similar in form to the curves made by plotting enzyme activity against the hydrogen ion concentration of the solutions in which they acted. While these fungi are not molds, the hydrogen ion concentration required to inhibit their growth is similar to that required to inhibit the growth of molds. Von Euler and Svanberg (35) reported that *Aspergillus niger* was inhibited by a P_H of 9.0. Webb (38) states that successively increasing the concentrations of hydrogen ions from neutral to P_H=3.1 to 2.8 favorably influences germination of the spores of *Aspergillus niger, Penicillium cyclopium, Botrytis cinerea, Fusarium* (sp) and *Lenzites sepiaria.* The germination was influenced by the hydrogen ion concentration varying with the organism.

Duggar, Severy and Schmitz (6) report that *Aspergillus niger* grew well in a natural mangold decoction of P_H=4.5 but produced a heavier mat when the media was brought to P_H=6.

It has been found that bacteria, molds and even higher plants bring about changes in the reaction of the medium in which they grow. With certain media and specific organisms the change is toward acidity and a definite end point is reached at which the action ceases. Some investigators have considered this end point as the limiting or critical P_H value for the specific organism studied. Others simply mention the changes as interesting phenomena while many consider them as means of differentiating between species. Duggar, Severy and Schmitz (6) point out

that the saprophytic *Aspergillus niger* shifted the P_H toward a more acid reaction while the parasitic *Gloeosporium (Glomerella) gossypii* shifted it toward a more alkaline reaction. The same authors in another paper (9) report that *Aspergillus niger* inoculated into decoctions of bean, turnip, prune and potato with P_H values of 4.0 shifted the reaction to P_H=3.0. *Macrosporium commune* and *Glomerella (Glocosporium) gossypii* generally induced a pronounced shift toward alkalinity to as much as P_H=6 to 8. *Penicillium expansum* developed acidity when sugar was added but alkalinity in natural decoctions.

It is evident from the foregoing discussion of results secured by other investigators that little work on the effect of hydrogen and hydroxyl ion concentrations on mold growth has been accomplished.

EXPERIMENTAL.

PLAN OF EXPERIMENTS

Since it was the object of these experiments to determine the hydrogen ion, hydroxyl ion and salt concentrations that would inhibit the growth of molds, series of flasks containing culture media were made up of varying degrees of acidity and alkalinity and with varying amounts of the following salts:

KCl	K_2SO_4	K_2CO_3
NaCl	Na_2SO_4	Na_2CO_3
$CaCl_2$	$CaSO_4$	$CaCO_3$
$MgCl_2$	$MgSO_4$	$MgCO_3$

The acidity, alkalinity and salt concentrations used will be given in the discussion of results. It was necessary to run a large number of series of acid, alkali and salt solutions in order to check determinations of the inhibiting point. In all, records were kept of 4,652 flasks of solutions and 894 determinations were made of hydrogen ion concentration. New series could not be planned until the results of the previous series were available. The work was started with 12 molds which had been previously isolated and identified and were in the collection in the laboratory of the Department of Soil Chemistry and Bacteriology at the University of California. During the war, while the author was absent, all but three of these died. Four more cultures were secured from Dr. Waksman at New Jersey and the series started again. The work done with the 12 cultures will be referred to only briefly in the paper since work with them was not completed. However, some of the data secured with them are interesting, since the hydrogen ion concentration of the solutions was determined both before inoculation and after the molds had ceased growing.

Most of the results reported are from the growth of the seven molds, *Mucor glomerula, Fusarium bullatum, Fusarium oxysporium, Aspergillus oryzae, Aspergillus terricola, Penicillium variabile* and *Penicillium italicum.*

METHODS

Czapek's solution modified by additions of HCl, KOH and salts was used as the medium, made up as follows:

$NaNO_3$	2.0	gms.
K_2HPO_4	1.0	"
KCl	.5	"
$MgSO_4$	.5	"
$FeSO_4$	0.1	"
Cane sugar	30.0	"
Water (distilled)	1,000	c. c.

The molds were grown on this medium in 250 c. c. Erlenmeyer flasks of pyrex and non-sol glass. They were incubated in most cases from 12 to 14 days. This corresponds to Waksman & Cook's (37) recommendation. It was found that they had reached an apparent maximum as far as could be observed by a simple visual examination in that time. In the series to determine optimum reaction for growth they were incubated from four to six weeks. Incubation was at room temperature, about 23° C. This temperature was fairly uniform during the months that steam heat was used since the steam is controlled by thermostats, but at other times it was somewhat variable.

Inoculation was accomplished at first with some difficulty, the mycelium being so tough that it was very hard to secure sufficient material. Later, when each series was started, the molds were introduced into flasks of sterile media containing about ten grams of washed gravel between one-eighth and one-fourth inch in diameter. When they were to be used for the inoculation of a new series, these flasks were well shaken and a loopful of the suspension of mycelium and spores thus produced was used as the inoculum. Fresh cultures were usually used. This method proved very satisfactory. In no case was there a failure in inoculation, contamination very seldom occurred and a uniform amount of inoculum for each mold was obtained thruout a whole series. Such a method was necessary since in some series as many as 600 flasks were used. In the first several series molds were inoculated into four flasks of each reaction. These checked so closely that in later series only duplicate inoculations were made.

The reaction of the media was adjusted by the use of normal HCl and KOH. J. F. Clark (6) has shown that the chlorine ion

has practically no injurious effect and that HCl while most highly ionized is the least toxic of the mineral acids in the same hydrogen ion concentration. Hence HCl was used because the inhibiting effect would be due almost entirely to the hydrogen ions formed. KOH was used because the potassium ions in the concentrations used would not be present in sufficient quantities to exert any harmful influence and the inhibiting effect would be due to the hydroxyl ions.

The P_H values produced in the medium by the additions of varying amounts of acid are shown by the curve in fig. 1. As made up, this is a composite curve of the results of hydrogen ion determinations in several series of solutions.

In the salt studies, Baker's analyzed chemicals were used in nearly all cases. When not, Merck's "Blue Label" or "Highest Purity" chemicals took their place. The salts were added in molecular proportions with variations in most cases of one-eighth of a mole per liter between different solutions. In the case of K_2CO_3 and Na_2CO_3 variations of one sixty-fourth of a mole per liter were made in the case of some molds which were very sensitive to these salts.

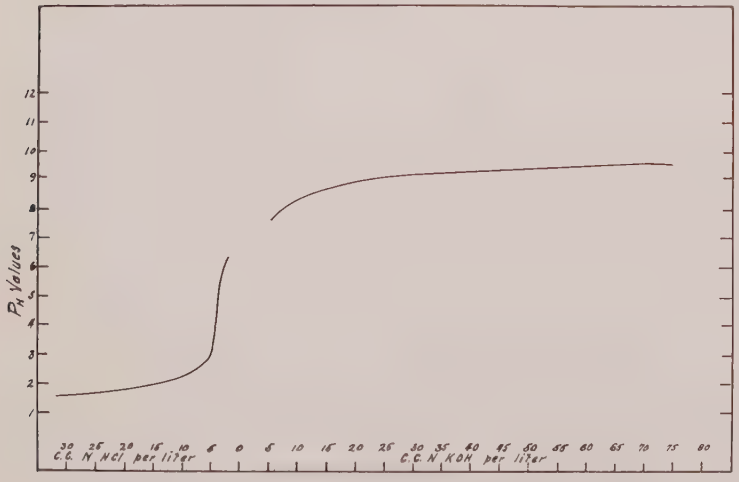


Fig. 1. The P_H Values Produced in the Medium by the Additions of Varying Amounts of Acid and Alkali.

Growth in the general series to determine the inhibiting action was observed only by the eye and recorded as follows:

No. 7—Luxuriant growth. (The best growth observed.)
No. 6—Good growth. (Not as much growth as in No. 7, but nearly so.)
No. 5—Fair growth. (Probably two-thirds as much growth as in No. 6.)
No. 4—Slight growth. (About two-thirds as much growth as in No. 5.)
No. 3—Very slight growth. (About one-half as much growth as in No. 4.)
No. 2—Very, very slight growth. (About one-half as much growth as in No. 3.)
No. 1—Very, very, very slight growth. (Barely perceptible growth).
No. 0—No growth.

When weighings were made, the mycelium was transferred to a dried, weighed filter paper in a funnel, thoroly washed with distilled water, dried in an oven at 105° C. cooled in a dessicator and paper and mold weighed together. This weight minus the weight of the paper is given as the weight of the mold growth.

The hydrogen ion and hydroxyl ion concentrations were determined electrometrically, using an apparatus similar to the one described by Hildebrand (6) and a hydrogen electrode similar to the one used by Sharp and Hoagland (6, (1)). An accurate Weston voltmeter reading from 0 to 1.5 volts by .01 of a volt and capable of being estimated to within .002 of a volt, was used with a sensitive Leeds and Northrup galvanometer and two rheostats, one 12 inches and the other 20 inches long. This equipment allowed readings to be made to within .002 volts. The calomel electrode was made up with KCl recrystallized three times, calomel made electrolytically after Clark (6) and mercury purified by thoro shaking with a solution of mercurous nitrate acidified with nitric acid. Five liters of normal KCl solution were made up and saturated with the calomel and kept for use in the electrode. The electrode was checked with an electrode made up by the Botany department and gave exactly the same readings with two different solutions. Hydrogen was prepared electrolytically from a 20 percent KOH solution and purified by passing thru a tube of platinized asbestos heated by a coil of resistance wire, and then thru two wash bottles of pure water. The hydrogen electrodes were shaken mechanically in a shaker devised by the author and operated by a small motor.

In making up media, two extra flasks of each different reaction were prepared and sterilized along with the others. The hydrogen ion concentrations were determined on each of these flasks after sterilization, as Norton (6) has shown that sterilization may affect the reaction of some media, especially when alkaline. The hydrogen ion concentrations in duplicate flasks were nearly always exactly alike and very seldom varied by as much as .05 of a P_H value.

TABLE I—GROWTH OF TWELVE MOLDS IN ACID SOLUTIONS OF VARYING NORMALITY

Mold	N 5	N 10	N 20	N 50	N 100	N 200	N 300	N 400	N 500
Penicillium variabile		0	0	4	6	6	6	6	6
Asidia cylindrospora	0	0	0		4	0	4	4	0
Alternaria (sp)	0	0	0	0	0	4	6	6	6
Fusarium	0	0	0	0	0	0	0	0	4
Dematium (sp)	0	0	0	0	0	4	6	6	6
Fusariuom solani	0	0	0	0	4	6	6	6	6
Aspergillus terricola	0	0	0	0	6	6	6	6	6
Cephalosporium	0	0	0	0	0	0	4	4	4
Mucor glomerula	0	0	0	0	0	6	6	6	6
Penicillium desiscens	0	0	0	0	6	6	6	6	6
Rhyzopus oryzae	0	0	0	0	0	5	4	4	4
Mucor sylvaticus	0	0	0	0	0	5	5	5	5

0—No growth.
4—Slight growth
5—Fair growth.
6—Good growth.

All determinations are expressed in P_H values, a term so widely used that it is unnecessary to explain it here. Voltmeter readings were changed to P_H values by the use of Schmidt and Hoaglands tables (6).

INHIBITING EFFECT OF HYDROGEN IONS

In the beginning of this work, very little idea could be obtained as to how much acidity molds in general would withstand. Consequently the 12 molds were inoculated into series of flasks of media varying in acidity so as to be equivalent to N/5, N/10, N/20, N/50, N/100, N/200, N/300, N/400 and N/500 HCl solutions. After incubation it was found that none of the 12 grew in concentrations higher than N/50 and that all but one grew in N/300. Table I shows a record of the growths obtained.

A new series of N/50, N/100, N/200 and N/300 HCl was then started with results shown in table II. The hydrogen ion concentrations of the four different solutions used in this series were determined and at the end of the incubation period the P_H values of the solution under each mold were found. The changes brought about by the growth will be discussed in a later section. The growth in the two series did not check entirely altho some similarity was shown. The differences might be due to varying hydrogen ion concentrations since this determination was not made on the first series.

After this second series it became evident that 12 molds were too many to work with, so in the third series the number was cut down to six, selected for their wide variations in growth.

In the third series no hydrogen ion determinations were made but an attempt to narrow the differences in acidity was made,

TABLE II—GROWTH OF TWELVE MOLDS IN ACID SOLUTION OF VARYING NORMALITY

Mold	N P_H=1.71 / 50	N P_H=2.03 / 100	N P_H=2.60 / 200	N P_H=2.96 / 300
Penicillium variabile	0	6	6	6
Absidia cylindrospora	0	0	0	0
Alternaria (sp)	0	0	4	6
Fusarium	0	0	0	4
Dematium (sp)	0	0	0	4
Fusarium solani	0	0	5	6
Aspergillus terricola	4	6	6	6
Cephalosporium (sp)	0	0	0	0
Mucor glomerula	0	0	6	6
Penicillium desiscens	5	6	6	6
Rhyzopus oryzae	0	0	0	0
Mucor sylvaticus	0	3	6	6

0—No growth.
3—Very slight growth.
4—Slight growth.
5—Fair growth.
6—Good growth.

so that concentrations of N/25, N/50, N/75, N/100, N/125, N/150, N/175, N/200, N/250, and N/300 HCl were used. The growth in these solutions is given in table III.

After the war only *Mucor glomerula, Penicillium variabile* and *Aspergillus terricola* survived. To these were added *Fusarium bullatum, Aspergillus oryzae, Fusarium oxysporium* and *Penicillium italicum,* secured from Dr. Waksman.

A series of solutions equivalent to N/50, N/66, N/100, N/200 and N/300 HCl was started with these seven molds and results as shown in table IV were obtained. The P_H values of these solutions were determined and are given in the table. From this series an indication of the concentration of HCl needed to inhibit the growth of each organism was obtained and thereafter each organism was inoculated into series of flasks with small variations on either side of the apparent end point.

TABLE III—GROWTH OF SIX MOLDS IN ACID SOLUTIONS OF VARYNG NORMALITY

Mold	N / 25	N / 50	N / 75	N / 100	N / 125	N / 150	N / 175	N / 200	N / 250	N / 300
Penicillium variabile	0	6	6	6	—					
Alternaria (sp)	—	—	—	—	—	6	6	6	6	6
Aspergillus terricola	0	4	6	6		—	—	—		—
Mucor glomerella	—	—	—	0	6	6	6	6	—	—
Penicullium desiscens	4	6	6	6	—	—	—	—	—	—
Fusarium solani	—	—	0	6	6	6	6	6	—	—

0—No growth.
4—Slight growth.
6—Good growth.
— —Not inoculated into these concentrations.

TABLE IV—THE GROWTH OF SEVEN MOLDS IN ACID SOLUTIONS

Mold	N P_H=1.81	N P_H=2.06	N P_H=2.28	N P_H=2.95	N P_H=4.12
	50	66	100	200	300
Mucor glomerula	0	0	0	0	6
Fusarium bullatum	0	0	0	4	6
Aspergillus oryzae	6	6	6	6	6
Fusarium ozysporium	0	4	4	6	6
Penicillium variabile	6	6	6	6	6
Aspergillus terricola	6	6	6	6	6
Penicillium italicum	0	0	2	6	6

0—No growth.
2—Very, very slight growth.
4—Slight growth.
6—Good growth.

The data from the several series of cultures to determine the effect of hydrogen ion concentration on the growth of each mold are shown graphically in fig. 2. Some molds required more series than others to fix the end point and a rather wide variation in the amount of acidity withstood by the various molds is evidenced.

The limiting hydrogen ion concentrations are given in table V.

It will be noted that in some series the results are widely at variance with the results of the majority. This is true in the case of all the molds but more so with *Fusarium bullatum* than with the others.

In an attempt to find the cause for these variations four experiments were conducted.

Since the temperature at which the molds were incubated varied somewhat, an experiment was run to determine the effect of different temperatures on the relation between hydrogen ion concentration and mold growth. In this work three different temperatures were roughly maintained, 33° C in a regulated incubator, 23° C in the room which was fairly constant, it being winter when steam heat regulated by a thermostat was in use,

TABLE V—THE LIMITING HYDROGEN ION CONCENTRATION FOR SEVEN MOLDS

Organism	Limiting P_H value
Mucor glomerula	3.2 to 3.4
Aspergillus oryzae	1.6 to 1.8
Aspergillus terricola	1.6 to 1.8
Penicillium variabile	1.6 to 1.8
Penicillium italicum	1.9 to 2.2
Fusarium bullatum	2.0 to 2.2
Fusarium ozysporium	1.8 to 2.2

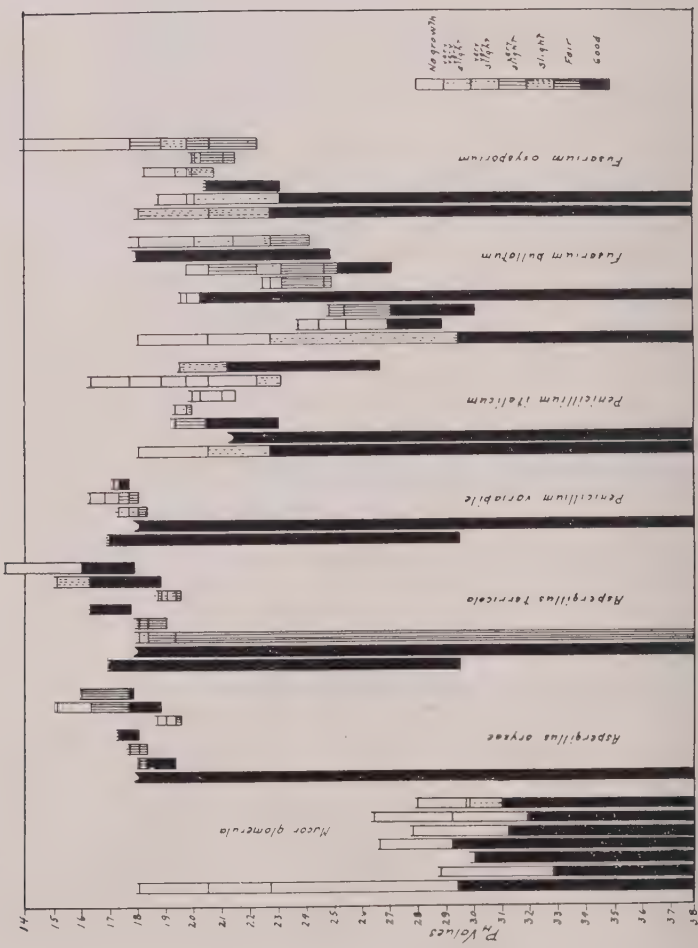


Fig. 2. Growth of Molds in Acid Solutions of Various PH Values.

and 13° C in a box lined with asbestos and placed just inside and against a window. The temperature in the box was observed several times a day and never varied over four degrees either up or down. Two molds were used in the experiment and the results are given in table VI.

It is evident that temperature has considerable influence on the effect of hydrogen ions on growth. Clark (5) states that an increase in temperature increases the effect of hydrogen ion concentration in the case of *Bacterium coli.* Coleman (8) gives the optimum temperature for fungal activity as 30° C. In a study of enzymes Palitzsch and Walbum (6, (2)) found that as the temperature was increased the optimum reaction changed toward the neutral point and Compton (6) confirmed this. In the work reported here a change either below or above the room temperature increased the effect of hydrogn ion concentration so that the limiting P_H value was shifted toward the neutral. It is possible then that the differences in temperature at which the series were incubated caused the variations in limiting hydrogen ion concentrations.

In another experiment cultures two weeks old and three months old were inoculated into series of flasks of media with the same P_H values. The older culture grew in the same hydrogen ion concentrations as the younger one showing that the age of the culture used for inoculum did not affect its resistance to acidity.

In order to determine the effect of the amount of inoculum added on the relationship between the P_H value of the solution and mold growth a series of flasks of media of varying hydrogen ion concentrations were inoculated with different amounts of a suspension of mycelium and spores. The mold used was a *Fusarium* and the data obtained are given in table VII.

TABLE VI—THE EFFECT OF TEMPERATURE ON THE RELATION BETWEEN HYDROGEN ION CONCENTRATION AND MOLD GROWTH

Mucor glomerula										
P_H of solution			4 68	3.89	3 65	3 30	3 30	3.18	2 99	2 94
Growth at 13° C			6	6	6	0	0	0	0	0
Growth at 23° C			6	6	6	6	6	0	0	0
Growth at 33° C			6	6	6	0	0	0	0	0
Fusarium oxysporium										
P_H of solution	2.15	2 05	1 94	1 89	1 83	1 81	1.78	1 70	1 64	1.62
Growth at 13° C	5	5	4	0	0	0	0	0	0	0
Growth at 23° C	6	6	6	6	5	0	0	0	0	0
Growth at 33°	6	5	5	0	0	0	0	0	0	0

0—No growth
6—Good growth.
5—Fair growth.
4—Slight growth.

TABLE VII—THE GROWTH OF FUSARIUM IN SOLUTIONS OF VARYING PH VALUES AS AFFECTED BY DIFFERENT AMOUNTS OF INOCULUM

PH values	2.33	2.16	2.00	1.98	1.93	1.83	1.64
Growth with 1 loopful	6	6	5	4	1	0	0
Growth with 2 loopfuls	6	6	5	5	1	0	0
Growth with 1 c.c.	7	7	7	6	6	5	1

7—Luxuriant growth.
6—Good growth.
0—No growth.
5—Fair growth.
4—Slight growth.
1—Very, very slight growth.

It is seen from these results that large amounts of inoculum shift the end point of the inhibiting action. Kopeloff (22) states that an increase in the number of spores inoculated into a medium is responsible for a proportional increase in ammonification. However, this would not be caused by the same factor which allows growth in a more acid medium when more spores are introduced. It may be that the larger amounts of inoculum were sufficient to change the hydrogen ion concentration altho it seems hardly probable that one c.c. of neutral medium, as slightly buffered as is this solution, added to 50 c.c. of medium should neutralize the effect of .2 c.c. of normal HCl. It is possible that the spores in solutions too acid to allow visible growth make a start to grow. In some work to be reported later in the paper it is shown that the growth of molds in an acid solution shifts the reaction toward neutrality, a very small growth producing some change. Thus in the case of the larger amounts of inoculum there may be sufficient growth due to the very large numbers of spores to shift the reaction sufficiently to allow the molds to make a visible growth in solutions where smaller numbers of spores could not cause such action. The variations in the effect of hydrogen ion concentration between different series may thus be due to different amounts of inoculum used. Altho a loopful of the spore suspension was added in each case, a great variation in spore production in the cultures used from time to time very probably occurred and the same quantity of inoculum may have introduced vastly different numbers of spores.

In some cases the mycelium was weighed and different weights of growth were obtained in similar solutions. The flasks in which the molds were grown varied slightly in size, so an experiment to determine the effect of varying the surface area of the media on the weight of mold produced was carried out. Two molds were grown in flasks of widely different sizes on the same quantity of media of the same composition. The results are given in table VIII.

TABLE VIII—RELATION OF AREA OF SURFACE OF MEDIA TO GROWTH

Mold	Area of surface	Wt. of growth
Aspergillus terricola	32 sq. cm.	176 mgs.
Aspergillus terricola	32 sq. cm.	191 mgs.
Aspergillus terricola	95 sq. cm.	207 mgs.
Aspergillus terricola	95 sq. cm.	207 mgs.
Penicillium desiscens	26 sq. cm.	410 mgs.
Penicillium desiscens	26 sq. cm.	429 mgs.
Penicillium desiscens	95 sq. cm.	563 mgs.
Penicillium desiscens	95 sq. cm.	544 mgs.

It will be seen that tripling the surface area does not allow a corresponding increase in growth and it is apparent that the small variations in size of flasks cannot appreciably affect the weight of mycelium produced.

From these experiments it seems that the variations in the limiting hydrogen ion concentration in different series may be due either to differences in the temperature at which they were incubated or to differences in the number of spores introduced. The limiting P_H values for growth, however, were taken from the series which represent the results obtained in the majority of the series, the series at wide variance with the majority not being considered as representative.

It is evident that even under the same conditions the end point for growth is not sharp, in the same series a change in P_H of .2 to .5 being required to cause a change from good growth to no growth, the growth passing from good to slight, very slight, etc., with smaller changes in P_H. It is true, however, that in some cases a very slight change in P_H will cause a change from exceedingly slight growth to no growth, as is evidenced in the case of *Penicillium italicum* growing at P_H 1.98 and showing no growth at P_H 1.94, or *Penicillium variabile* growing at 1.78 and not at 1.74, or *Aspergillus terricola* showing both growth and no growth at P_H 1.64, the change between flasks apparently being too small for detection with the apparatus used. *Aspergillus oryzae* also grew at P_H 1.96, but not at 1.94. There are several other examples of this sharp end point between exceedingly slight growth and no growth in individual series.

The end point is apparently a hydrogen ion concentration factor. In one series *Mucor glomerula* grew in a solution containing 5.2 c.c. of N HCl per liter but failed to grow in one with 5.4 c.c., while in another series it grew when 8 c.c. of the same acid had been added per liter. In the case of *Fusarium oxysporium,* which is less sensitive, growth occurred in a media with 15.2 c.c. of N HCl per liter but not in one with 16.0 c.c., whereas in another case it grew in a solution with 18 c.c. per liter. The

other molds show similar variations. Hydrogen ion determinations on these solutions, however, show that altho these solutions in which growth occurred contain varying quantities of acid, their P_H values are very nearly alike.

The critical P_H values of these molds are low, the lowest 1.6 to 1.8 and the highest 3.2 to 3.4. This is very low as compared to the limiting P_H of other organisms and ferments as determined by many workers. A few results may be mentioned here for comparison:

The critical P_H values for all the molds, except *Mucor glomerula*, are much lower than for any of the organisms, enzymes or plants noted above. Clark and Lubs (6, (2)) give the reactions of the more acid solutions produced by bacteria and other organisms as follows:

Product	P_H values
Cider vinegar	3.1
White vinegar	2.6
Corn silage juice	3.7
Wines	2.8-3.8
Beers	3.9-4.7

If the reactions of these solutions represent the limiting P_H values for the organisms producing them, it is evident that the molds are much more resistant than even these highly resistant forms.

TABLE IX—RESULTS OBTAINED BY OTHER INVESTIGATORS

Organism or enzyme	Critical P_H	Authority
Azotobacter	5.9	Gainey (6)
Azotobacter	5.5–5.6	Waksman (6, (1))
Azotobacter	6.4	Fred & Davenport (6)
Rhizobium leguminosarium (alfalfa and sweet colver)	4.8	Fred & Davenport (6)
Rhizobium leguminosarium (garden peas, field peas, vetch)	4.6	Fred & Davenport (6)
Rhizobium leguminosarium (red clover, beans)	4.1	Fred & Davenport (6)
Rhizobium leguminosarium (lupine)	3.1	Fred & Davenport (6)
Bacillus coli	4.3–5.3	Clark & Lubs (6, (1))
Pneumococcus	7.0	Dernby & Avery (6)
Diphtheria bacillus	6.3	Bunker (4)
Bacillus mesentericus	5.0	Cohn, Walbach & Henderson (6)
Bacterium typhosum	5.0	Schoenhol- & Meyer (6)
Bacterium typhosum	4.0	Fennel & Fisher (6)
Spongospora subterranea	4.8	Gillespie (6, (4))
Yeasts	2.94	Boas & Leberle (6)
Yeasts	2.5–2.7	Clark & Lubs (6, (2))
An orchid, *Habenaria blepharigloltis* growing at	3.5	Wherry (6, (2))
Barley seedlings decidedly injured at	3.5	Hoagland (6, (1))
Alfalfa	3.6	Joffe (21)
Seedlings of corn, wheat, alfalfa and soy beans	2.96	Salter & McIlvaine (6)
Trypsin	5.1	Clark (6, (2))
Invertase	3.6	Clark (6, (2))
Erepsin	5.1	Clark (6, (2))
Protease	5.1	Clark (6, (2))
Maltase	6.2	Clark (6, (2))
Urease	5.5	Clark (6, (2))
Pancreatic amylase	4.0	Sherman, Thomas & Baldwin (6)
Malt amylase	2.5	Sherman, Thomas & Baldwin (6)
Amylase from *Aspergillus oryzae*	2.6	Sherman, Thomas & Baldwin (6)

TABLE X—GROWTH OF SEVEN MOLDS IN ALKALINE SOLUTIONS

Mold	20 c.c. N KOH per L	40 c.c. N KOH per L.	60 c.c. N KOH per L.	80 c.c. N KOH per L.	100 c.c. N KOH per L.
Mucor glomerula	0	0	0	0	0
Fusarium bullatum	6	5	1	0	0
Aspergillus oryzae	1	0	0	0	0
Fusarium oxysporium	5	1	0	0	0
Penicillium variabile	3	0	0	0	0
Aspergillus terricola	4	1	0	0	0
Penicillium italicum	5	0	0	0	0

0—No growth.
1—Very, very, very slight growth.
3—Very slight growth.
4—Slight growth.
5—Fair growth.
6—Good growth.

INHIBITING EFFECT OF HYDROXYL IONS

In order to determine the approximate amount of alkalinity the seven molds would withstand, a series of solutions was made up containing 20, 40, 60, 80 and 100 c.c. of normal KOH per liter into which the molds were inoculated. The results obtained are given in table X.

From these results a new series with concentrations more suitable for each mold was planned. After this several series of solutions were made up, sterilized, their P_H values determined and then inoculated and incubated.

Detailed records of the results for each mold are shown graphically in fig. 3.

The hydroxyl ion concentration which caused an inhibition of growth could not be determined as definitely as the hydrogen ion concentration doing the same, the end point apparently not being so definite. However, the P_H values in this direction which stopped growth, within a somewhat wider range than in the direction of acidity, were determined. They are given in table XI.

TABLE XI—THE LIMITING HYDROXYL ION CONCENTRATION FOR SEVEN MOLDS

Mold	Limiting P_H values
Mucor glomerula	8 7– 9.2
Aspergillus oryzae	9 0– 9 3
Aspergillus terricola	9 0– 9 3
Penicillium variabile	10 1–11 1
Penicillium italicum	9.1– 9 3
Fusarium bullatum	9.2–11 2
Fusarium oxysporium	9 2–11.1

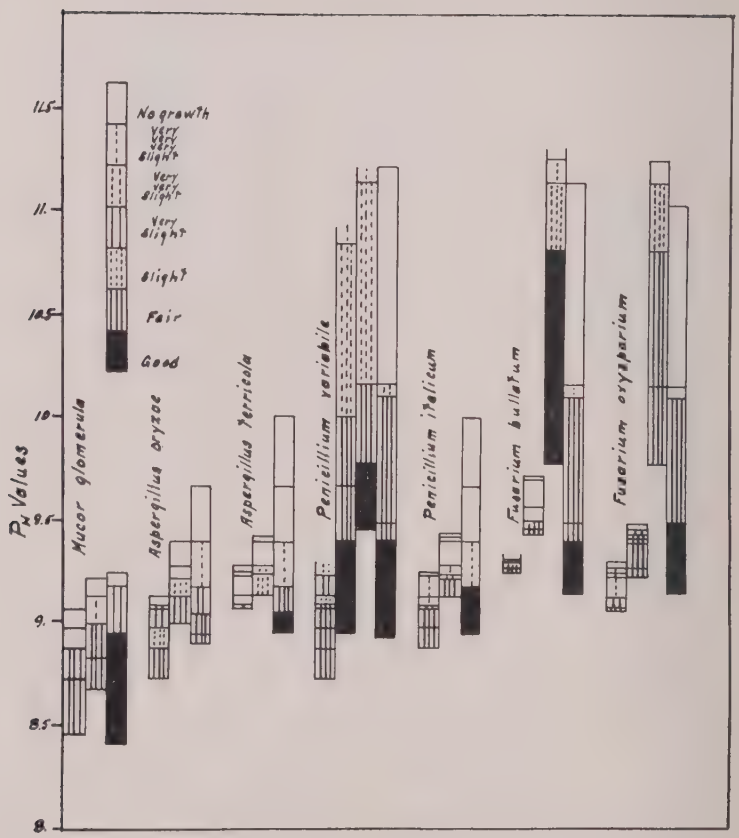


Fig. 3. Growth of Molds in Alkaline Solutions of Various PH Values.

The first notable feature of these results is that the hydroxyl ions are much more toxic than the hydrogen ions. P_H 3.2 which inhibits the growth of *Mucor glomerula* is equivalent to an hydrogen ion concentration of about .6x10-3 or .0006 gms. of ionized hydrogen per liter. P_H 8.7, which inhibits it in the alkaline series, is equal to an hydroxyl ion concentration of about 5.1x10-6 or .0000051 gms. of OH ions per liter. Thus the hydroxyl ion concentration inhibiting growth is approximately 1-120 of the concentration of the hydrogen ions preventing development. In the case of *Penicillium variabile* the ratio between the hydroxyl ion and hydrogen ion concentrations in-

TABLE XII—RESULTS OBTAINED BY OTHER INVESTIGATORS

Organism or Enzyme	Critical P_H	Authority
Pneumococcus	8.3	Dernby & Avery (6)
Diphtheria bacillus	8.2	Bunker (4)
Bacterium typhosum	8.6	Schoenholz & Meyer (6)
Bacillus subtilis	9.0	Itano (6, (2))
An orchid *Cypridedium candidum* grew at	8.0	Wherry (6, (2))
Barley seedlings seriously injured by	9.4	Hoagland (6, (1))
Pancreatic amylase	10.0	Sherman, Thomas & Baldwin (6)
Malt amylase	9.0	Sherman, Thomas & Baldwin (6)
Amylase from *Penicillium niger*	8.0	Sherman, Thomas & Baldwin (6)

hibiting growth is about as 1:240, while with *Fusarium oxysporium* it is about 1:975. The greater toxicity of the OH ions was pointed out by J. F. Clark (6) in studying the effect of titratable acidity and alkalinity on mold growth and has been referred to by several investigators. Another outstanding feature is that *Mucor glomerula* which was most sensitive to acidity is also most sensitive to alkalinity.

From the number of references, it seems that more investigators have been interested in the effect of acidity than alkalinity. However, to compare the alkalinity withstood by molds to that inhibiting other organisms, reference will be made to results given by other workers.

Comparing the limiting P_H for the molds with the above reported results, it is apparent that molds can grow in more alkaline solutions than can the bacteria and plants and are inhibited at about the same reaction as is enzyme activity.

THE INHIBITING EFFECT OF VARIOUS SALTS

In studying the effect of salts on these seven molds KCl, NaCl, $CaCl_2$. $2H_2O$, $MgCl_2$. $6H_2O$, K_2SO_4 Na_2SO_4. $10H_2O$, $CaSO_4$. $2H_2O$. $MgSO_4$. $7H_2O$, K_2CO_3, Na_2CO_3, $CaCO_3$ and $MgCO_3$, combinations of four basic ions with three acid ions, were used.

In the first series $CaSO_4$, $CaCO_3$ and $MgCO_3$ were eliminated since all the molds grew in saturated solutions of these salts, which of course are practically insoluble. In a later series K_2SO_4 was eliminated for the same reason.

As stated in the section on methods, the salts were added in molecularly equivalent quantities.

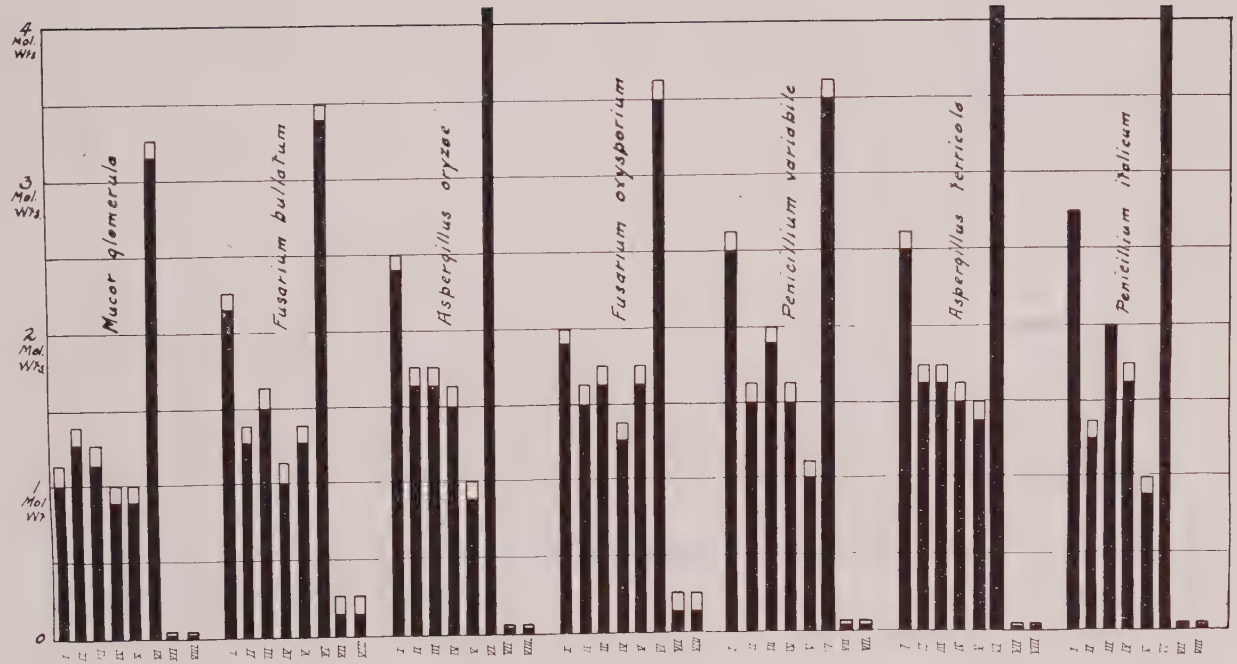
The inhibiting concentrations obtained are given in table XIII and are shown graphically for each mold in fig. 4 and for each salt in fig. 5.

In the case of *Penicillium italicum* the inhibiting amounts of KCl, $MgCl_2$ and $MgSO_4$ were not definitely determined, growth occurring in these salt solutions with greater concentrations than

TABLE XIII—INHIBITING SALT CONCENTRATIONS

	Highest Mol Conc. for Mold Growth	Inhib. Mol. Conc.	Inhib. Conc. Gms. per liter.*
Mucor glomerula			
KCl	1	1⅛	83.88
NaCl	1¼	1⅜	80.41
$CaCl_2.2H_2O$	⅞	1	110.99
$MgCl_2.6H_2O$	1⅛	1¼	119.05
$Na_2SO_4.10H_2O$	⅞	1	142.07
$MgSO_4.7H_2O$	3⅛	3¼	391.30
K_2CO_3	1-128	1-64	2.16
Na_2CO_3	1-128	1-64	1.97
Fusarium bullatum			
KCl	2⅛	2¼	167.76
NaCl	1¼	1⅜	80.41
$CaCl_2.2H_2O$	1	1⅛	124.86
$MgCl_2.6H_2O$	1½	1⅝	154.70
$Na_2SO_4.10H_2O$	1¼	1⅜	195.36
$MgSO_4.7H_2O$	3⅜	3½	421.40
K_2CO_3	⅛	¼	34.54
Na_2CO_3	⅛	¼	31.50
Aspergillus oryzae			
KCl	2⅜	2½	186.40
NaCl	1⅝	1¾	102.34
$CaCl_2.2H_2O$	1½	1⅝	180.31
$MgCl_2.6H_2O$	1⅝	1¾	166.60
$Na_2SO_4.10H_2O$	⅞	1	142.07
$MgSO_4.7H_2O$	4⅛+		496.65
K_2CO_3	1-32	1-16	8.63
Na_2CO_3	1-32	1-16	7.87
Fusarium oxysporium			
KCl	1⅞	2	149.12
NaCl	1½	1⅝	95.03
$CaCl_2.2H_2O$	1¾	1⅞	152.57
$MgCl_2.6H_2O$	1⅝	1¾	166.60
$Na_2SO_4.10H_2O$	1⅝	1¾	248.64
$MgSO_4.7H_2O$	3½	3⅝	436.45
K_2CO_3	¼	¼	34.54
Na_2CO_3	⅛	¼	31.50
Penicillium variabile			
KCl	2½	2⅝	195.72
NaCl	1½	1⅝	95.03
$CaCl_2.2H_2O$	1½	1⅝	180.31
$MgCl_2.6H_2O$	1⅞	2	190.48
$Na_2SO_4.10H_2O$	1	1⅛	161.84
$MgSO_4.7H_2O$	3½	3⅝	436.45
K_2CO_3	1-32	1-16	8.63
Na_2CO_3	1-32	1-16	7.87
Aspergillus terricola			
KCl	2½	2⅝	195.72
NaCl	1⅝	1¾	102.34
$CaCl_2.2H_2O$	1½	1⅝	180.31
$MgCl_2.6H_2O$	1⅝	1¾	166.60
$Na_2SO_4.10H_2O$	1⅜	1½	213.10
$MgSO_4.7H_2O$	4⅛+		496.65+
K_2CO_3	1-128	1-64	2.16
Na_2CO_3	1-64	1-32	3.94
Penicillium italicum			
KCl	2¾+		205.04
NaCl	1¼	1⅜	80.41
$CaCl_2.2H_2O$	1⅝	1¾	194.18
$MgCl_2.6H_2O$	2+		190.48+
$Na_2SO_4.10H_2O$	⅞	1	142.07
$MgSO_4.7H_2O$	4⅛+		496.65+
K_2CO_3	1-128	1-64	2.16
Na_2CO_3	1-64	1-32	3.94

*Grams per liter of salts without water of crystalization.



I KCl, II NaCl, III $MgCl_2$, IV $CaCl_2$, V Na_2SO_4, VI $MgSO_4$, VII K_2CO_3, VIII Na_2CO_3

Fig. 4. The Molecular Amounts of Various Salts that Inhibit the Growth of Seven Molds. Salts Compared for Each Mold.

for the other molds. In the case of *Aspergillus oryzae* and *Aspergillus terricola* the concentration of $MgSO_4$ inhibiting growth was not reached even tho 4⅛ gram molecular weights per liter of solution were used.

Mucor glomerula is here again more sensitive to all the salts than any of the other molds. A variation in the toxicity of the individual salts to the different molds is evident; that is, KCl is not as toxic to some molds as it is to others. Also, a wide variation in the toxicity of the different salts to individual molds was found, for instance, Na_2SO_4 is three and one-fourth times as toxic to *Mucor glomerula* as $MgSO_4$ in molecular amounts.

From fig. 5, it appears that molecule for molecule K_2CO_3 and Na_2CO_3 are by far the most toxic of the salts studied and about equally so. This is no doubt due to the hydrolysis of the salts with consequent alkalinity. Thus the media with 1/32 of a mole of Na_2CO_3 per liter had a P_H value of 9.26 and with the same amount of K_2CO_3, 9.23, which values are near the limiting P_H for all the molds. Na_2SO_4 is next in order and $MgSO_4$ is the least, with NaCl, $MgCl_2$ and $CaCl_2$ about equally toxic, while KCl is between these last three and $MgSO_4$.

Thus they would rank in order of least toxic to most toxic, in molecularly equivalent amounts, $MgSO_4$, KCl, $MgCl_2$, NaCl, $CaCl_2$, Na_2SO_4, Na_2CO_3, and K_2CO_3.

If the basis of grams per liter of solution is used leaving out of consideration in figuring the water of crystallization, they would rank in ascending order of toxicity, $MgSO_4$, Na_2SO_4, KCl, $MgCl_2$, $CaCl_2$, NaCl, K_2CO_3 and Na_2CO_3.

This does not correspond with Greaves' (13) ranking for these salts in their effect on ammonification in soils. He gives Na_2CO_3, K_2CO_3, $MgSO_4$, NaCl, $MgCl_2$, KCl, Na_2SO_4, and $CaCl_2$ as their order from least to greatest toxicity. Lipman (24) ranks the sodium salts in their effect on ammonification differently, giving NaCl as most toxic, Na_2SO_4 much less so and Na_2CO_3 only slightly toxic at very high concentration. Singh (32) gives even a different ranking to these sodium salts as affecting ammonification, NaCl most toxic Na_2CO_3 less and Na_2SO_4 very much less. Brown and Johnson (3) give the salts in order of greatest to least toxicity as $NaHCO_3$, Na_2CO_3 and Na_2SO_4.

In their effects on nitrification Lipman (25) ranks the salts from least toxic to most toxic in the order of Na_2SO_4, NaCl and Na_2CO_3, while Brown and Hitchcock (2) give Na_2CO_3 and $NaHCO_3$ as least toxic with Na_2SO_4 next and NaCl most toxic. For the nitrogen fixing organisms Lipman and Sharp (26) rank them in the same order as Lipman does for nitrification. This is more nearly in the order of their effect on molds and as Lipman points out it is more like their effect on higher plants.

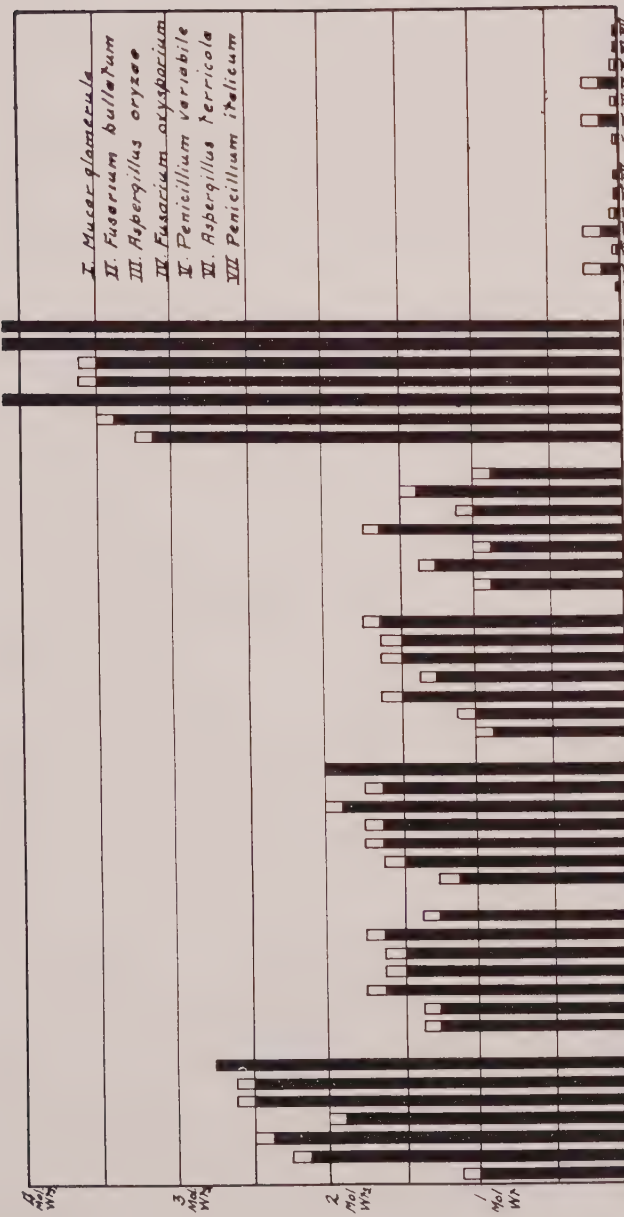


Fig. 5. The Molecular Amounts of Various Salts that Inhibit the Growth of Seven Molds. Molds Compared for Each Salt.

Harris [15] ranks them from least toxic to the germination and growth of crops to their greatest toxicity in the order $MgSO_4$, Na_2SO_4, K_2CO_3, Na_2CO_3, $MgCl_2$, KCl, $CaCl_2$ and NaCl. Headden [17] gives $MgSO_4$, Na_2SO_4, Na_2CO_3 as the order from least to greatest toxicity to sugar beets.

For barley, Hilgard [18] ranks the salts as Na_2SO_4 least toxic, NaCl next and Na_2CO_3 most toxic, while Pittman [29] gives the salts as Na_2SO_4, Na_2CO_3 and NaCl from least to greatest toxicity to wheat seedlings and this order is checked by Singh [32].

Absolute amounts of these salts that were toxic are all stated by these authors in parts by weight in soils, hence they are not in any way comparable with the amounts used in the solutions in these experiments and are not given.

It is plainly seen that the salts rank differently in their effect on the different soil processes and that even with the same process different investigators have not agreed on their relative toxicity.

If any comparison can be made with general soil figures, the molds will withstand immensely greater amounts of salts than will crops. Taking Harris' [16] results, which show that soils containing .5 percent of soluble salts, where the larger part is chlorides, carbonates or nitrates, and 1.0 percent where sulfates predominate, are unsuitable for crop production without reclamation, it figures out that the soil solution in such soils would contain only two to four percent of salts. This is on the basis of an average soil, taking 2,000,000 pounds per acre, with 20 to 25 per cent of moisture; that is, the 450,000 to 500,000 pounds of water would contain, if all the salts were in solution, from 10,000 to 20,000 pounds of salts. The molds here reported grew in from one percent of Na_2CO_3 to 48 percent of $MgSO_4$ with an average for the chlorides of about 15 percent.

THE OPTIMUM REACTION FOR THE GROWTH OF MOLDS

While studying the effect of reaction it seemed that to complete the study, the optimum reaction for growth should be determined.

Accordingly a series of solutions with reactions varying from acid to alkaline was made up with P_H values of 1.78, 2.71, 3.75, 4.28, 5.78, 6.27, 6.54, 6.63, 7.05, 8.39, 8.71 and 9.06. Each of the molds was inoculated in duplicate into flasks of each concentration in which they would grow, and incubated six weeks. At the end of this time the weight of the growth in each flask was determined.

The weights thus obtained are shown graphically in fig. 6.

As can be seen, no definite or conclusive results were obtained

in this series, most of the molds showing very erratic behaviors and exhibiting two optima widely separated.

Consequently another series was started and this time three flasks of each concentration were inoculated with each mold. Apparently the distilled water used was acid, so that the additions of acid made did not produce solutions of regularly varying P_H as expected, several of the acid solutions being of nearly the same P_H values. The solutions used had P_H values of 1.83, 2.35, 2.40, 2.48, 2.57, 4.26, 6.54, 8.71, 9.47 and 10.09.

The results from this series are given graphically in fig. 7. These results are almost as erratic as those in the previous series.

It is to be regretted that the solutions were not highly buffered since it was later found that the growth of the molds had changed the reaction to a very marked degree.

The optimum reaction for these molds lies apparently between a wide range of P_H values. Fennel and Fisher (6) remark, in regard to *B. typhosus* and *B. paratyphosus*, that "these have wide ranges of P_H in which they will grow and therefore an ill defined optimum." Certainly these molds have a wide range in which they will grow and their optima are very ill defined. Cohen and Clark (7) in speaking of *B. bulgaricus*, *B. coli*, *B. aerogenes* and *B. proteus* say that wide zones of P_H were shown where growth remains near the maximum but on the borders of these zones slight changes in P_H produce large effects. This is quite true of the molds. Altho they have a wide zone in which their growth remains near the maximum, at the alkaline edge a change of .7 of a P_H value means in most cases a change from good growth to very little if any growth.

As many investigators have shown, most bacteria and plants have an optimum reaction for growth near or slightly above the neutral point. Just a few may be mentioned in table XIV.

TABLE XIV RESULTS OBTAINED BY OTHER INVESTIGATORS

Organism or Enzyme	Optimum P_H	Authority
Diphtheria bacillus	6.5–7.5	Bunker (4)
B cholerae	6.2–8.0	Fennel & Fisher (6)
B dysenteriae	6.3 7.8	Fennel & Fisher (6)
Meningococcus	7.6	Fennel & Fisher (6)
Gonococci	7.6	Fennel & Fisher (6)
B. radicicola (alfalfa)	7.2–7.72	Fred & Loomis (11)
Pneumococcus	7.0 8 3	Dernby & Avery (6)
Pancreatic amylase	7.0	Sherman, Thomas & Baldwin (6)
Malt amylase	4.4–4.5	Sherman, Thomas & Baldwin (6)
Amylase from *Aspergillus niger*	4.8	Sherman, Thomas & Baldwin (6)
Barley seedlings	5.1	Hoagland (6, (1))
Wheat seedlings	5.94	Salter & McIlvaine (6)
Soy bean seedlings	5.94	Salter & McIlvaine (6)
Alfalfa seedlings	5.94	Salter & McIlvaine (6)
Corn seedlings	5.16	Salter & McIlvaine (6)

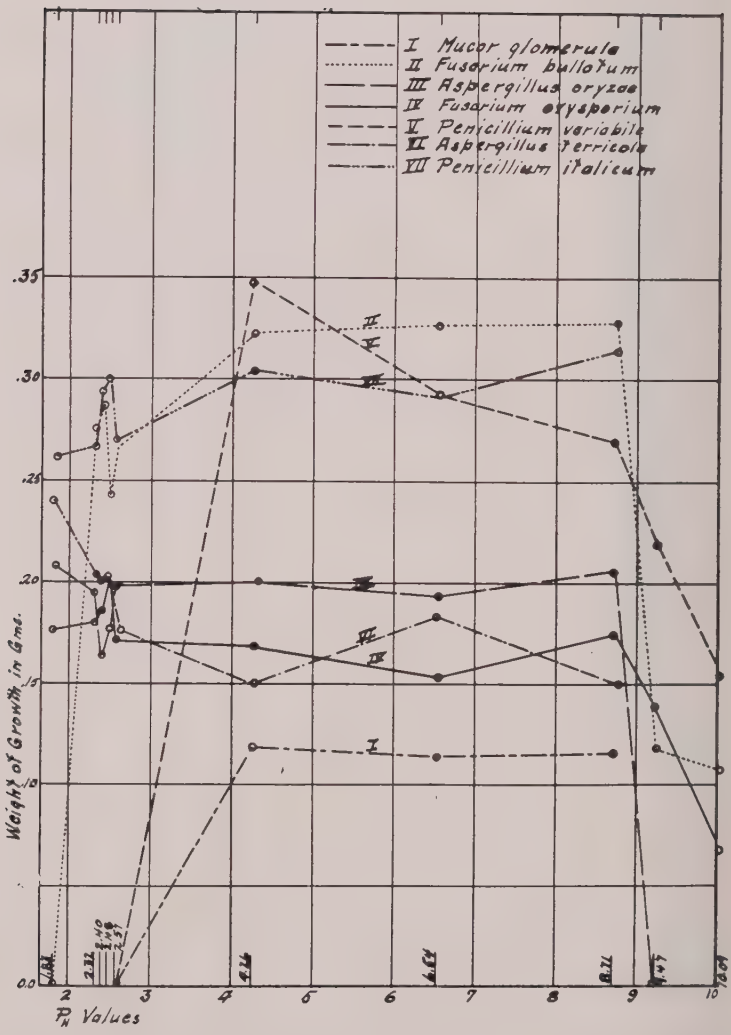


Fig. 6. Relation of Weight of Growth of Molds to the Initial PH Values of the Media.

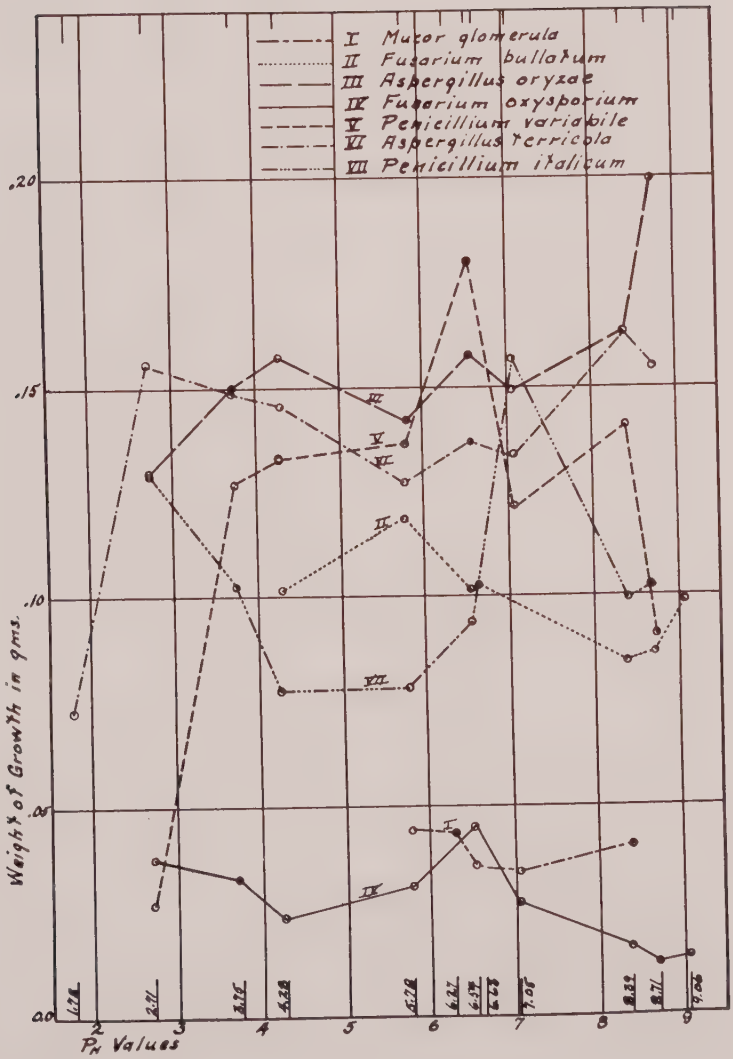


Fig. 7. Relation of Weight of Growth of Molds to the Initial PH Values of the Molds.

From the data in these two series the optimum reaction for the molds lies more to the acid side of the neutral point than toward the alkaline, practically all of them making nearly a maximum growth as low as P_H=2, while they are cut off quite sharply at P_H=8.7.

CHANGES IN REACTION BROUGHT ABOUT BY MOLD GROWTH

With the second series of molds grown it was discovered that considerable changes in reaction were induced in the solution by the growth. In every case, where the molds grew, a change toward the neutral point and even in many cases over to an alkaline reaction was brought about and this change was in proportion to the growth. This was after only two weeks incubation. In the flasks where good growth occurred each mold seemed to reach a more or less definite end point. Only slight changes were found where no growth occurred. The results of this experiment are given in table XV and include the data for the 12 molds originally used.

Later a series with the seven molds was made up in which each mold was inoculated in duplicate into flasks with neutral solutions and flasks with solutions whose acidity was near the maximum in which the molds would make a good growth. These were incubated three months. Some evaporation took place, but not sufficient to influence the results obtained appreciably.

The P_H values obtained before and after growth are given in table XVI.

It will be noted that the reaction of all the solutions was changed toward a more alkaline value except the neutral solution with *Fusarium bullatum* and all, except with this mold, were made alkaline. The two solutions in the case of each mold were brought nearer together in P_H values but the end point reached was quite different in the two solutions. In no case did the molds reach the P_H values which were found to inhibit their growth in the studies on the effect of hydroxyl ions.

At the end of the incubation period of six weeks in the second series to determine the optimum reaction for mold growth, the solution in each flask was used for hydrogen ion determinations. The data thus obtained are given in table XVII and are shown graphically in fig. 8.

Here again it is seen that the molds bring about a change in reaction.

In this case we have molds growing in both acid and alkaline solutions and in both cases the reaction is changed toward the neutral. With the acid solutions, wherever good growth oc-

TABLE XV—CHANGES IN PH VALUE OF SOLUTIONS CAUSED BY THE GROWTH OF SEVEN MOLDS*

PH at beginning	*Penicillium variabile* Growth; PH at 2 wks.		*Aosidia cylindrospora* Growth; PH at 2 wks.		*Alternaria* (sp) Growth; PH at 2 wks.		*Fusarium* (sp) Growth; PH at 2 wks.		*Dematium* (sp) Growth; PH at 2 wks.		*Fusarium solani* Growth; PH at 2 wks.		*Aspergillus terricola* Growth; PH at 2 wks.		*Mucor glomerula* Growth; PH at 2 wks.		*Penicillium desiscens* Growth; PH at 2 wks.		*Mucor sylvaticus* Growth; PH at 2 wks.	
1 71	—	1 81	—	1 94	—	1 94	—	1.98	—	1 83	—	1.98	S	1.84	—	1.78	—	1 78	—	1.93
	S	1 89	—	1 96	—	1 98	—	1 91	—	1.67	—	1 98	S	1.84	—	1.74	F	4.38	—	1.96
2 03	G	5.22	—	2 18	—	2 18	—	2 20	—	2.15	—	2 18	G	8 76	—	2 10	G	8 34	vvs	2 18
	G	4 82	—	2 18	—	2 18	—	2.16	—	2.11	—	2 18	G	8 76	—	2.11	G	7 32	vs	2.45
2 60	G	6 88	—	2 62	S	2.79	—	2 67	—	2.62	F	8 54	G	8.91	G	8 71	G	7 83	G	8 20
	G	6 91	S	2 64	S	2.67	—	2 64	vvs	2 60	F	8 55	G	8 91	G	8 37	G	8 34	G	8.37
2 96	G	6 85	—	2 96	G	8 49	S	3 19	S	3 75	G	8 59	G	8.99	G	8 37	G	8.27	G	8 59
	G	6 91	S	2 96	G	8 49	S	3 16	vs	3 09	G	8 71	G	8 98	G	7.42	G	8 12	G	8 59

**Cephalasporium* (sp) and *Rhyzopus oryzae* made no growth in these solutions so are not included
— No growth.
vvs Very, very slight growth.
vs Very slight growth
S Slight growth.
G Good growth.

TABLE XVI—CHANGES IN P_H VALUES OF SOLUTIONS CAUSED BY THE GROWTH OF SEVEN MOLDS

Mold	P_H at start	P_H at 15 wks.
Mucor glomerula	6.78	7.96
		7.91
	6.09	8.10
		8.20
Fusarium bullatum	6.78	5.56
		5.34
	3.01	5.46
		5.07
Aspergillus oryzae	6.78	8.08
		7.90
	1.81	5.78
		5.71
Fusarium oxysporium	6.78	8.74
		8.74
	2.15	8.06
		7.96
Penicillium variabile	6.78	8.45
		7.78
	1.91	6.88
		6.24
Aspergillus terricola	6.78	8.89
		8.89
	1.91	6.54
		6.54
Penicillium italicum	6.78	8.23
		8.25
	2.05	2.74*
		2.16

**Penicillium italicum* did not make a good growth at P_H 2.05.

curred, the reaction was so changed that it became alkaline. With the alkaline solutions, the alkalinity was reduced, the reactions of the two coming very nearly to the same P_H value.

However, the variation in the final reactions was of such magnitude that a characteristic end point for each of the several molds could not be approximated. If the molds were compared as to the end reactions produced in any one initial P_H value, a characteristic end point for each would be found, but if these end points were compared with the end points produced at other initial P_H values they would be found to differ.

Changes of as much as 6.6 in P_H values were caused when acid solutions were made alkaline and 2.0 when alkaline solutions were decreased in alkalinity. Others who have investigated the changes brought about in the reaction of media by bacterial and plant growth have found similar results.

Itano (6, (2)) reports *B subtilis*, *Streptococcus erysipelatis* and *Streptococcus lacticus* to have changed media. Since these organisms would not grow in such wide extremes of reaction the

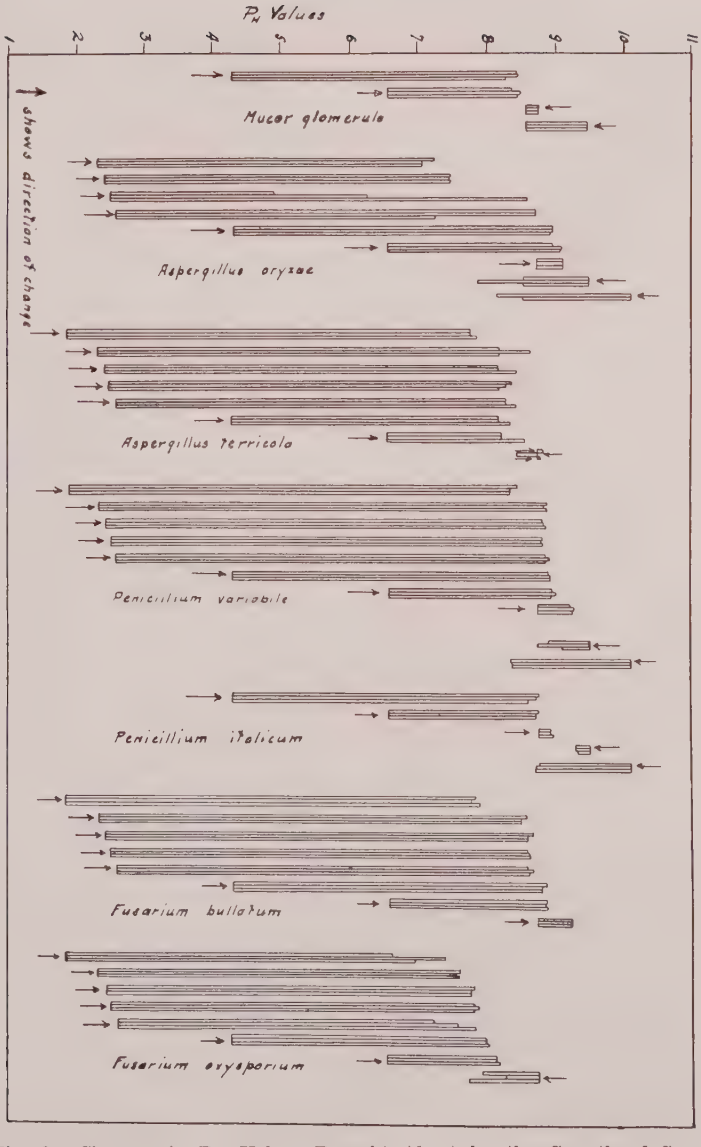


Fig. 8. Changes in PH Values Brought About by the Growth of Seven Molds.

TABLE XVII—CHNGES IN PH VALUES OF SOLUTIONS CAUSED BY GROWTH OF SEVEN MOLDS

PH Values at start	*Mucor glomerula*		*Fusarium bullatum*		*Aspergillus oryzae*		*Fusarium oxysporium*		*Penicillium variabile*		*Aspergillus terricola*		*Penicillium italicum*	
	Growth in gms.	PH at 6 wks.	Growth in gms.	PH at 6 wks.	Growth in gms.	PH at 6 wks.	Growth in gms.	PH at 6 wks.	Growth in gms.	PH at 6 wks.	Growth in gms.	PH at 6 wks.	Growth in gms.	PH at 6 wks.
1 83					.2020	7.73	.1569	8.40			.2625	7.79	.2721	6.59
					.2155	7.73	.1923	8.32			.2190	7.74	.2570	7.37
					.2100	7.81	.1806	8.32			.2411	7.88	.2550	6.93
2.32			.2880	7.25	.2000	8.15	.1850	8.84			.2230	8.54	.2540	7.59
			.2587	7.05	.1830	8.35	.2003	8.79			.2175	8.55	.2022	7.57
			.2680	7.05	.2006	8.12	.1556	8.83			.1741	8.45	.2840	7.59
2.40			.2546	7.47	.1462	8.10	.2020	8.77			.2091	8.64	.2990	7.81
			.3020	8.42	.1640	8.10	.1915	8.79			.1859	8.49	.3040	7.71
			.3145	8.45	.1764	8.42	.1653	8.81			.2053	8.59	.2748	7.73
2 49			.1330	4 87	.1771	8.34	.2040	8.79			.2105	8.57	.3210	7.81
			.2424	6.24	.1895	8.23	.1968	8.76			.2209	8.57	.2810	7.88
			.3500	8.55	.1630	8.18	.2028	8.79			.1806	8.59	.2981	7.81
2.57			.2298	8.66	.1860	8.30	.1748	8.84			.1805	8.57	.3090	7.21
			.3200	8.62	.2100	8.30	.1646	8.88			.1621	8.66	.2200	7.59
			.2477	7.25	.1985	8.44	.1750	8.84			.1900	8.59	.2800	7 81
4.26	.1103	8.42	.3018	8.91	.2091	8.17	.1623	8.88	.3298	8.74	.1391	8.83	.2940	7.96
	1080	8.42	.3761	8.91	.2704	8.17	.1689	8.89	.3739	8.69	.1390	8.79	.3050	8.00
	1369	8 23	.2898	8.89	.1903	8.35	.1782	8.89	.3354	8.57	.1740	8.77	.3150	8.01
6.54	.1167	8 37	.3269	8 94	.1822	8.20	.1472	8.93	.2884	8.71	.1905	8.84	.2891	8.12
	.1117	8.44	.3255	9.06	.1923	8.18	.1507	8.99	.3020	8.69	.1830	8.84	.3000	8.12
	1170	8.40	.3316	9.05	.2080	8.55	.1672	8.91	.2881	8.69	.1785	8.86	.2997	8.15
8.71	.1233	8 55	.3435	9.06	.1964	8.84	.1801	9.21	.2784	8.91	.1489	9.21	.3276	7.90
	.1108	8.55	.3432	9.06	.2054	8.39	.1750	9.23	.2741	8.91	.1546	9.21	.3130	8.25
	1160	8 55	.2989	9.06	.2170	8.77	.1717	9.23	.2600	8 94	.1550	9.21	.3050	7.73
9 47	.1233	8 55	.1380	8.50			.1560	8.86	.1981	9.25				
	1108	8.55	.0800	7.84			.1265	8.71	.2405	9.25				
	1160	8 55	.1370	8.50			.1327	9.08	.2230	9.26				
10.09			0150	8 10			.0695	8.32	.1253	8 71				
			.0230	8.50			.0671	8.39	.1370	8.71				
							0730	8.34	.2070	8.67				

changes brought about by them are not so great, yet they are very similar to these mold results, acid solutions being made alkaline and alkaline ones being reduced in alkalinity. He also found no definite end points, the reactions caused in solutions starting with various P_H values, varying.

Avery and Cullen (6, (1)), however, found that they could differentiate between human and bovine types of *Streptococcus haemolyticus* by the end point reached in the growth of the types. This checked out with a large number of strains.

Ayres (6) found two definite zones of P_H values brought about by *streptococci* from different sources, and Ayres, Johnson and Davis (6) state that in a dextrose, yeast, peptone medium pathogenic *streptococci* reach a final P_H of 5.4-6 while a non-pathogenic strain reached P_H 4.5-4.9.

In studying *B coli*, Clark (6, (4)) found those from animal sources to reach P_H=4.77, while those from grain stopped at P_H=5.09. He found, however, that on different media different end points were reached.

Zeller, Schmitz and Duggar (39) state that, in the case of wood destroying fungi, the shifting of the hydrogen ion concentration due to metabolism depends both upon the fungus and the medium in which they are grown.

Avery and Cullen (6, (2)) report that cultures of *pneumococcus* reach a P_H of 5.0 if .4 of a percent of fermentable carbohydrate are present, but if it is not present growth ceases at P_H=7.

Clark (6, (6)) states that several cultures of *Bacillus bulgaricus* brought the reaction of various media to P_H=3.8.

Clark and Lubs (6, (1)) found that bacteria of the *colon aerogenes* family could be differentiated by the use of indicators, since one group gave an end reaction of about P_H 4.5 while another one gave P_H=6.2.

Lord and Nye (6) found that, in a one percent solution of glucose, cultures of *pneumococcus* reached a final P_H value of 5.1.

Fred and Davenport (6) give changes brought about by various strains of *Rhizobium leguminosarium* but, since they started with practically a neutral media, very little change was brought about. *Azotobacter* changed the media to an acid reaction of P_H=5.1.

Waksman and Joffe (6) in studying *actinomycetes* conclude that, with media of different hydrogen ion concentration, the *actinomycetes* tend to change the reaction to an optimum.

Salter and McIlvaine (6) state that plants tend to adjust the P_H toward a slightly acid reaction.

Hoagland (6, (2)) found that barley and bean seedlings tend to change the solutions toward neutrality. He found that the

amount of the different salts absorbed from the solution affected the reaction.

Duggar, Severy and Schmitz (6) showed that *Aspergillus niger* grown on plant decoctions caused a shift in P_H from 4 to 3. *Macrosporium commune* and *Glomerella* (*Glocosporium*) *gossypii* generally induced a pronounced shift toward the alkaline from P_H 4 to P_H=6 to 8. *Penicillum* developed acidity when sugar was added to the media but in natural decoctions produced alkalinity. They point out that the saprophytic *Aspergillus* shifts the reaction toward acidity while the parasitic *Glocosporium* brought about a more alkaline reaction.

Boas and Leberle (6) noted that the final P_H values with *Aspergillus fumigatus* lie within a wide range of from 1.56 to 5.7. For yeasts a narrower range of from P_H 2.94 to 3.80 was found.

From these few references it is evident that all organisms so far studied are capable of changing the reaction of the medium in which they grow. However, the magnitude of the changes caused by organisms other than the molds is not so great, due to the rather narrow limits within which they grow. Like the molds here discussed, all seem to change media in the direction of neutrality if the media to start with are some distance either side of that point.

The reasons, or rather the mechanisms, of this change are not explained. It is accepted that the carbohydrates may be converted into acids and thus cause an acid reaction. The use of the basic ions in such salts as KCl and K_2HPO_4 may cause some more acidity and the taking up of the acid radicles of the $NaNO_3$, $MgSO_4$ and K_2HPO_4 may give rise to alkaline reactions as pointed out by Hoagland (6, (2)). That the use of the nitrogen from $NaNO_3$ may cause alkalinity was demonstrated in a series by the author wherein the reaction of solutions originally containing 2 gms. and 4 gms. of $NaNO_3$ per liter were determined after *Mucor glomerula* had grown one week in them. At the start the reaction of both solutions was P_H=4.18. At the end the solution with 2 gms. gave a P_H of 6.46 and the one with 4 gms. P_H 6.85. It is evident that this can explain some of the alkalinity produced. It is difficult to explain, however, why an acid solution is changed toward alkaline while an alkaline solution is decreased in alkalinity.

From the results given it can hardly be concluded that the change is in the nature of a natural adjustment of the reaction toward an optimum since in nearly all cases the acid solutions were carried past the neutral point to an actual alkalinity, while it seems evident and has always been accepted that a slight acidity favored mold growth.

DISCUSSION

Sharp and Hoagland (6, (1)) report soils with as wide variations in P_H as 3.62 and 9.50. Gillespie (6,(2)) noted a soil with a P_H of 4.55 and another of 8.7. Russell (31) states that the extreme value for acid soils is P_H 4.4. Plummer (6) found a range from P_H 4.04 to P_H 9.68, and when a Cecil clay was treated with $(NH_4)_2SO_4$ it gave a P_H of 3.68. Morse (6) in studying the effect of fertilizers found one plot treated with $(NH_4)_2SO_4$ to give a P_H of 4.9. According to these investigations the lowest P_H was 3.62 and the highest 9.68.

From the results reported in this paper it is evident that the extreme acidity of P_H 3.62 is not sufficient to inhibit the growth of even the most sensitive molds. The highest alkalinity, P_H 9.68 might inhibit the growth of the more sensitive molds but not the majority of them. Such extreme reactions are found only under exceptional soil conditions and it may be concluded that as far as soil reaction is concerned, molds may play an important role in practically all of our soils.

Such reactions are toxic to most bacteria as reported by other investigators, so that Hall, Miller and Gimingham (14) may be correct in their statement that the decline in fertility of the acid plots may be attributed to the repression of the normal bacterial activities of the soil and the encouraging of the growth of molds. However, molds are generally considered to be more or less beneficial.

Hutchinson (19) has reported that the application of caustic lime to a soil causes a partial sterilization which lasts until the CaO has been transformed to $CaCO_3$. Hoagland and Christie (6) have shown that CaO may give rise in a soil to P_H values of from 8.56 to 10.82 when applied in quantities of 1,400 pounds per acre of 2,000,000 pounds or 10.16 to 12.45 when used at the rate of 5,600 pounds, according to the soil type used. The larger amounts give a reaction sufficient to inhibit the growth of even the most resistant molds. Hence Hutchinsons observations are quite easily conceivable.

It is apparent that fungi cannot be controlled by changing the soil reaction unless it be changed to a much greater extent than plants would survive.

The molds apparently will survive more acid, more alkali and a greater concentration of salts than any of the other organisms so far reported.

SUMMARY

The inhibiting effect of hydrogen ions, hydroxyl ions and salt concentrations on the growth of seven molds were studied. Also an attempt was made to determine the optimum reaction for their growth and the change in reaction of the media in which they have grown has been studied.

The critical P_H values, above and below the neutral point, for each of the seven molds were found to be as follows:

	Acid reaction Inhibiting growth	Alkaline reaction inhibiting growth
Mucor glomerula	P_H=3.2 to 3.4	P_H=8.7 to 9.2
Fusarium bullatum	P_H=2.0 to 2.2	P_H=9.2 to 11.2
Aspergillus oryzae	P_H=1.6 to 1.8	P_H=9.0 to 9.3
Fusarium oxysporium	P_H=1.8 to 2.0	P_H=9.2 to 11.1
Penicillium variabile	P_H=1.6 to 1.8	P_H=10.1 to 11.1
Aspergillus terricola	P_H=1.6 to 1.8	P_H=9.0 to 9.3
Penicillium italicum	P_H=1.9 to 2.2	P_H=9.1 to 9.3

The order of the salts from least toxic to most toxic, molecule for molecule, is $MgSO_4$ KCl, $MgCl_2$, NaCl, $CaCl_2$ Na_2SO_4, Na_2CO_3 and K_2CO_3. In the same order, according to grams per liter of media, they are $MgSO_4$, Na_2SO_4, KCl, $MgCl_2$, $CaCl_2$, NaCl, K_2CO_3 and Na_2CO_3. The molds will withstand greater concentrations of these salts with the possible exceptions of K_2CO_3 and Na_2CO_3, than other organisms studied.

An optimum reaction for their growth was not determined, since apparently they make a maximum growth in a very wide range of P_H values so that the optimum is a wide zone which lies more toward the acid than the alkaline side of the neutral point.

All the molds changed the reaction of media in which they grew, in one case a change of 6.6 in P_H was noted. The acid solutions were changed to alkaline in most cases and alkaline ones were made less alkaline. The change was not necessarily toward an optimum as was evidenced by the fact that the changes were carried from the acid past the neutral point to alkalinity whereas the optimum was apparently more toward the acid side.

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